An Axl-targeting Monoclonal Antibody that Inhibits Axl Activity and Potently Stimulates the Innate Immune Response

Diego Alvarado[1], Laura Vitale[2], Mike Murphy[1], Thomas O'Neill[2], Edward Natoli[1], Jay Lillquist[1], Linda Crew[1], Anna Wasiuk[2], Jeff Weidlick[2], Crystal Sisson[2], Jenifer Widger[2], Laura Mills-Chen[2], Andrea Crocker[2], Colleen Patterson[2], James Boyer[3], Eric Forsberg[3], April R. Baronas[3], Taylor M. Mathieu[3], Amelia C. Fields[3], Russell A. Hammond[3], Li-Zhen He[2], Joel Goldstein[2], Lawrence J. Thomas[3], Henry C. Marsh[3], Tibor Keler[2]

[1]Celldex Therapeutics, Inc., New Haven, CT 06511, [2]Hampton, NJ 08827 and [3]Fall River, MA 02723

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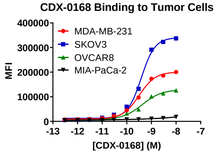
BACKGROUND

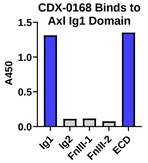
- Axl is a member of the TAM (Tyro3/Axl/MerTK) family of receptor tyrosine kinases and a negative regulator of innate immunity.
- Activation of Axl through its ligand Gas6 leads to suppression of myeloid cell activity, while its activation in tumor cells drives tumor growth, metastasis, and is associated with acquired resistance to targeted therapies, radiotherapy and chemotherapy.
- We describe a humanized IgG1 Axl-targeting monoclonal antibody (mAb), CDX-0168, that potently inhibits Gas6 binding and activation of Axl in tumor cell lines.
- CDX-0168 elicits a robust inflammatory response in human primary myeloid cells via an FcR-dependent mechanism, leading to T cell activation in mixed lymphocyte reactions.
- Administration of CDX-0168 to tumor cells co-cultured with human PBMCs leads to dose-dependent killing of Axl-expressing tumor cells *in vitro* and *in vivo*.
- The pleiotropic effects of Axl activation in cancer support combination of Axl-targeting agents with other targeted agents, either as drug combinations or as part of the same molecule.
- A prototype tetravalent bispecific (bsAb) antibody engineered to block both Axl and PD-L1 preserves full Axl and PD-L1 blockade and immune stimulatory activity.

CDX-0168 Potently Binds to Axl and Blocks Gas6 Binding

Axl Binding

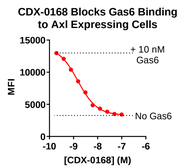


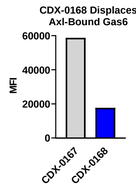
Monovalent CDX-0168 Binding to sAxl (Octet)	
K_D (nM)	0.705
kon (1/Ms)	6.08E+05
kdis(1/s)	4.29E-04
R^2	0.99

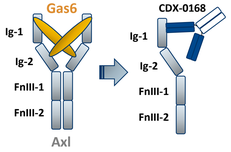


- CDX-0168 binds to tumor cell lines expressing varying amounts of Axl and recombinantly expressed sAxl with sub-nanomolar potency.
- Binding to purified Axl domains demonstrates binding to Ig1, the major Gas6 binding domain.

Gas6 Blocking



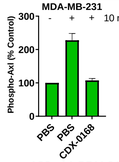


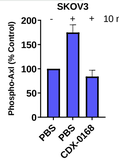


- CDX-0168 pre-incubation on Axl cells blocks fluorescently-labeled Gas6 binding and displaces pre-bound Gas6. CDX-0167 is an anti-Axl mAb that does not block Gas6.

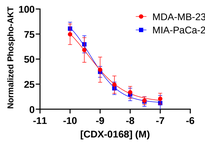
Inhibition of Gas6-Dependent Signaling

Axl Phosphorylation





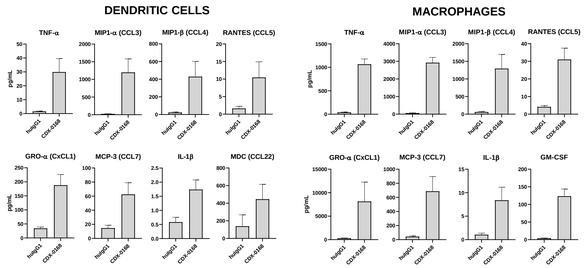
AKT Phosphorylation



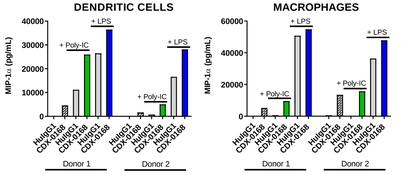
- 100 nM CDX-0168 blocks Gas6-dependent Axl phosphorylation in tumor cells and inhibits Gas6-dependent AKT phosphorylation.

Activation of Immune Responses in Myeloid Cells

Induction of Inflammatory Responses in Donor-Derived Human Myeloid Cells

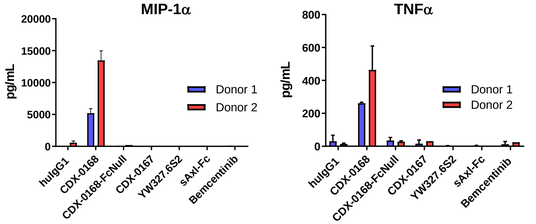


Enhancement of TLR Agonist Activity



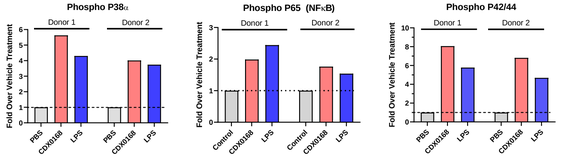
- Human monocytes were differentiated into macrophages with MCSF and dendritic cells (DCs) with IL-4 and GM-CSF.
- DCs and macrophages were treated with 100 nM CDX-0168 with or without 5 ug/mL poly-IC or 10 ng/mL of LPS for 24 hours.
- Cytokine and chemokine release were measured by ELISA or Luminex.

Immune Activation is FcR Dependent and Unique to CDX-0168



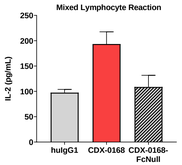
- **Cytokine induction by CDX-0168 requires binding to Fc receptors**
  - A CDX-0168 variant with impaired FcR binding (CDX-0168-FcNull) fails to elicit a cytokine response in human macrophages.
- **Immune activation is unique to CDX-0168 as other Axl inhibitors do not induce cytokine secretion**
  - Axl mAbs and Gas6 traps were added at 100 nM; bemcentinib was added at 1 uM.
  - CDX-0167 and YW327.6S2[a]: Axl inhibitory mAbs; sAxl-Fc: Gas6 "trap"; bemcentinib: Axl TKI. [a] Ye et al. Oncogene. 2010.

Activation of Inflammatory Signaling Pathways



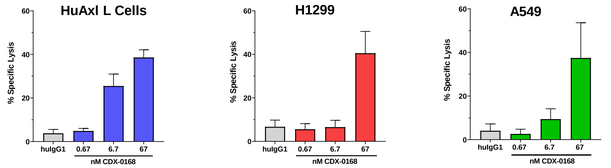
- Human donor macrophages were treated with control, 100 nM CDX-0168, or LPS for 1 hour. Phospho-proteins were quantified by western blot using an Odyssey CLx instrument and normalized to β-tubulin.

T Cell Activation in Mixed Lymphocyte Reactions



- CDX-0168 induces T cell activation in an FcR dependent manner in mixed lymphocyte reactions (MLR).
- CD4+ cells were co-incubated with allogeneic DCs and mAbs at 100 nM for 4 days. Secreted IL-2 levels were measured by ELISA.

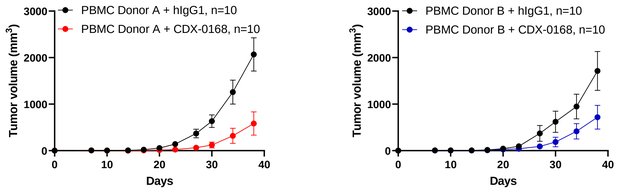
Tumor Cell Killing Through Effector Function

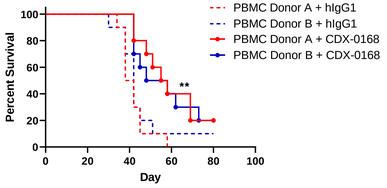


- Axl-expressing cells were co-incubated overnight with human donor PBMCs at a 75:1 ratio and treated with increasing doses of CDX-0168. Lysis was measured using CytoTox ONE™ assay from Promega. Dose-dependent cell killing was observed.

Antitumor Responses in Axl-Expressing Tumor Models

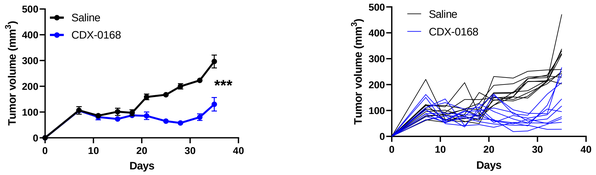
HuAxl L Cell Tumor Model





Murine L cells transfected to stably express human Axl were co-inoculated with human PBMCs into SCID mice. 10 mice were dosed i.p. with 300 ug of CDX-0168 or a control IgG1 mAb starting at days 1, 4, and 7 and with 150 ug at days 10, 14, 17 and 21. ** p-value < 0.01.

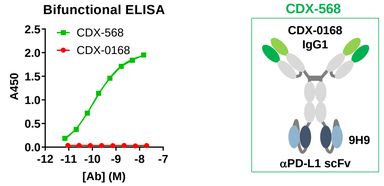
MDA-MB-231 Tumor Model

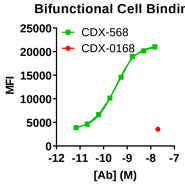


- Axl-expressing MDA-MB-231 breast tumor cells were co-inoculated with human PBMCs in SCID mice. 10 mice per group were dosed i.p. with 300 ug of CDX-0168 on day 0 and 100 ug subsequently q2wx4, and q1w thereafter.
- *** P=0.0002

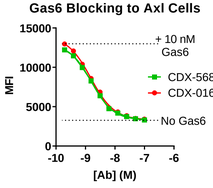
CDX-568: An Axl x PD-L1 Bispecific Antibody Based on CDX-0168

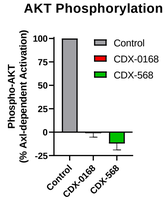
A CDX-0168-based bsAb co-targeting PD-L1 retains activity from each parental antibody

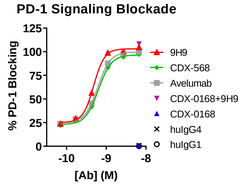




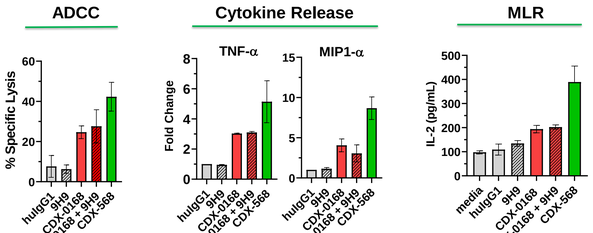
- CDX-568 is a tetravalent Axl x PD-L1 targeting bsAb that simultaneously binds both targets in ELISA and cell-based assays. Binding to human plate-coated or cell-expressed Axl is followed by detection with labeled soluble human PD-L1.







- CDX-568 blocks Axl and PD-1 signaling in cell-based assays with similar potency to each parental. PD-1 signaling was performed using a reporter assay (Promega).



- CDX-568 induces equal or better ADCC, cytokine release and T cell activation in MLR assays than combination of CDX-0168 with 9H9.

CONCLUSIONS

- **Directing a monoclonal antibody against a specific epitope in Axl can elicit antitumor activity via several mechanisms.**
  - CDX-0168 blocks Gas6 binding to Axl and downstream signaling.
  - Induces innate and adaptive immune activation *in vitro* in a FcR dependent manner.
  - Mediates cytotoxicity of Axl-expressing tumor cells *in vitro* and *in vivo* in the presence of human donor PBMCs.
- **Additional activities can be built into CDX-0168 through the generation of bispecific molecules.**
  - A prototype CDX-0168 x PD-L1 bsAb retains all the properties of the parental antibodies and demonstrates enhanced activity in immune activation assays.
  - Other combinations are under consideration.
- **Future efforts will focus around development of a multispecific molecule co-targeting Axl with a second immune modulator.**